Acta Pharm. Sci. Vol 61:(3), 2023
DOI: 10.23893/1307-2080.APS6115

Chemical constituents of *Ailanthus altissima* (Mill.) swingle leaves growing in Egypt and their antioxidant activity

Heba R. MOHAMED[1*], Eman A. EL-WAKIL[1], Maher M. EL-HASHASH[2], El-Sayed S. ABDEL-HAMEED[1]

1 Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Korniash El-Nile, 12411Warrak El-Hadar, Giza, Egypt

2 Department of Chemistry, Faculty of Science, Ain-Shams University, El-Khalifa El-Mamoun, 11566 Abasia, Cairo, Egypt

ABSTRACT

Ailanthus altissima Swingle (Simaroubaceae), the tree of heaven, is characterized by its vast pharmacological potential beside its rich and diverse phytochemical profile. The plant leaves dry powder was extracted with 85% aqueous methanol and the crude extract was then fractionated in sequence with petroleum ether, methylene chloride, ethyl acetate and *n*-butanol. The bioactive phytochemicals were separated from the ethyl acetate and the *n*-butanol fractions through column chromatography (CC) and quantified through HPLC-DAD. The chemical structure of the isolated pure compounds was identified through UV-Vis, ^{1}H-NMR, ^{13}C-NMR and TLC-MS spectroscopic analyses. The antioxidant activity of the pure compounds was estimated through their scavenging potential of DPPH$^{\cdot}$. Nine compounds were isolated and structurally elucidated as corilagin, astragalin, ellagic acid, gallic acid, isoquercetin, cynaroside, tellimoside, quercetin and luteolin. Ellagic acid, gallic acid and corilagin exhibited potent antioxidant activity. Corilagin derived from *A. altissima* can serve as potent natural antioxidant alternative.

Keywords: *Ailanthus altissima*, antioxidant, phenolics, HPLC-DAD, corilagin

INTRODUCTION

Ailanthus altissima Swingle had been used in the Chinese folk medicine as a remedy of many ailments such as cough, anemia, hemorrhage, diarrhea, dys-

*Corresponding Author: E-mail: hebaraafatp@sci.asu.edu.eg
ORCIDs:
Heba R. MOHAMED: 0000-0003-2114-775X
Eman A. EL-WAKIL: 0000-0003-1173-6024
Maher M. EL-HASHASH: 0000-0003-2550-8479
El-Sayed S. ABDEL-HAMEED: 0000-0002-0482-2170
(Received 24 Jan 2023, Accepted 8 Mar 2023)

entery, hemorrhoids as well as other gastric and intestinal disorders. Furthermore, many recent reports evaluated the medicinal value of *A. altissima* which was found to possess significant antimalarial, antiplasmodial, antiviral, antileukemic, antimicrobial, anti-inflammatory, cytotoxic and analgesic activities [1-4]. Many research concerned with studying the phytochemical constituents of the various parts of *A. altissima* and evaluating their medical properties. Different phytochemicals were isolated, purified and identified from *A. altissima* including quassinoids, alkaloids, sterols, terpenoids, flavonoids, phenolics, volatile components and lignans [5-10].

Presently, there is a growing concern toward finding out new and effective therapeutic compounds from natural resources, since they were found to possess equipotent pharmacological activities to the conventional synthetic drugs producing minimal side effects and less toxicity [11]. Plant derived natural products especially polyphenols are the best choice as they were reported to possess multiple potent biological activities against pathogenesis of many degenerative and chronic disorders [12].

Recently, the oxidative stress has been implicated in the induction of many maladies such as cardiovascular disorders, cancer, rheumatoid arthritis and neurological diseases. It occurs as a result of the disturbance of the oxidant-antioxidant homeostasis within the body when the oxidants mainly reactive oxygen species (ROS) exceeds the antioxidant defense ability of the body and hence they attack the various cell components; lipid, protein and DNA causing severe and irreversible oxidative destruction to them [13, 14]. So that both the natural and synthetic antioxidants play a vital role in the prevention of oxidative stress mediated diseases through their scavenging ability of ROS. As many recent researches discussed the toxicity and the side effects resulted from the use of synthetic antioxidant beside their high manufacturing costs, it becomes crucial to find out novel and potent antioxidants from natural sources [15].

The aim of the current study was to separate, purify and quantify the major chemical constituents in the two bioactive fractions derived from the methanol extract of *A. altissima* leaves through combination of various chromatographic techniques. Also, elucidation of the chemical structure of the isolated pure compounds via different spectroscopic tools. Moreover, investigation of the antioxidant potential of the isolated pure compounds via DPPH$^{\cdot}$ scavenging activity.

METHODOLOGY

Chemicals and reagents

DPPH (1, 1diphenyl-2-picryl hydrazyl radical) was purchased from Sigma–Aldrich (Steinheim, Germany); Organic solvents: methanol, petroleum ether (60-80°C), ethyl acetate, *n*-butanol, methylene chloride, sulphoric acid, acetic acid all were of analytical chemical grade obtained from El- Nasr pharmaceutical chemicals Co., Egypt; Adsorbents: Polyamide 6S (high purity grade) for column chromatography was purchased from Riedel-De Haën AG, Seelze-Hannover, Germany, Lipophilic sephadex LH-20, Silica gel 90 C_{18} - reversed phase for column chromatography and Readymade silica gel TLC cards (with fluorescent indicator 254 nm, layer thickness 0.2 mm, 20 x 20 cm aluminum cards were purchased from Sigma –Aldrich, Germany and Whatmann filter papers No. 1 and 3 mm for paper chromatography were purchased from England.

Plant material

The leaves of *Ailanthus altissima* were collected, in September 2017, from Orman Garden, Giza, Egypt. A plant specimen was identified by Mrs. Treaze Labib (a consultant of plant taxonomy at Agriculture Ministry and the ex-director of Orman Garden) and Mrs. Rehab Mohamed Eid (a botanist at Orman Garden Herbarium). A voucher specimen (No. 198 AC) was deposited in Orman Garden Herbarium. The collected fresh leaves were dried at ambient temperature away from the sunlight and were then ground to fine powder using electric mixer.

Extraction and fractionation processes

The plant leaves dry powder (2 Kg) was extracted with 85% aqueous methanol (7 L) at room temperature. The filtrate was further concentrated at reduced temperature and pressure via rotary evaporator (BUCHI, Germany) and the extraction process was repeated five times affording crude extract of uniform weight with yield 23.50 %. The crude methanol extract (470 g) was successively fractionated by partition using different organic solvents; petroleum ether, dichloromethane, ethyl acetate and *n*-butanol which were also evaporated under vacuum till complete dryness yielding 2.41, 38.16, 63.62, 162.00 and 143.00 g corresponding to petroleum ether, dichloromethane, ethyl acetate, *n*-butanol and aqueous fractions, respectively. The ethyl acetate and the *n*-butanol fractions derived from the methanol extract of *A. altissima* leaves possessed significant antioxidant and cytotoxic potential which could be attributed to their high content of phenolics and flavonoids [4] so that they were selected for subsequent chromatographic isolation procedures in order to separate and purify their active chemical constituents.

Chromatographic isolation of phenolic compounds from bioactive fractions

The ethyl acetate fraction (30 g) and the *n*-butanol fraction (50 g) were separately loaded into column chromatography (4×91cm) filled with polyamide. Elution was started with distilled water followed by gradient mixtures of water and methanol till pure methanol. Similar fractions on PC and TLC were collected. Promising groups were re-chromatographed on sephadex LH-20, RP-C18 silica gel and preparative paper chromatography and their products were monitored on PC and TLC. Four compounds were separated from the EtOAc fraction; Compound **1** was eluted with 100% water while compound **4** was eluted with 100% methanol. Compound **2** and **3** were eluted with 5-10% and 20-40% methanol, respectively. All four compounds were purified through applying over sephadex LH-20 except for compound **3** which was purified through preparative PC, after first purification with sephadex, using 15% AcOH as eluent system for PPC. Four other compounds were separated and purified from the *n*-BuOH fraction. Also, these compounds were purified on sephadex LH-20 using gradient elution of aqueous methanol. Compound **9** was further purified on RP-C18 after sephadex LH-20. The physical properties of each compound including color and retention factor (R_f) in three different eluting systems were detected. The used eluting systems were; 15% AcOH (S_1, PC), BAW (S_1, PC) and EtOAc: MeOH: H_2O (8: 1: 1) (S_3, TLC). Moreover, each compound was subjected to UV, NMR and TLC-MS spectral analyses as well as HPLC-DAD analyses to confirm its purity and detect its concentration in both the crude extract and the fraction. The scheme of extraction, fractionation and chromatographic separation is summarized in **Figure 1**.

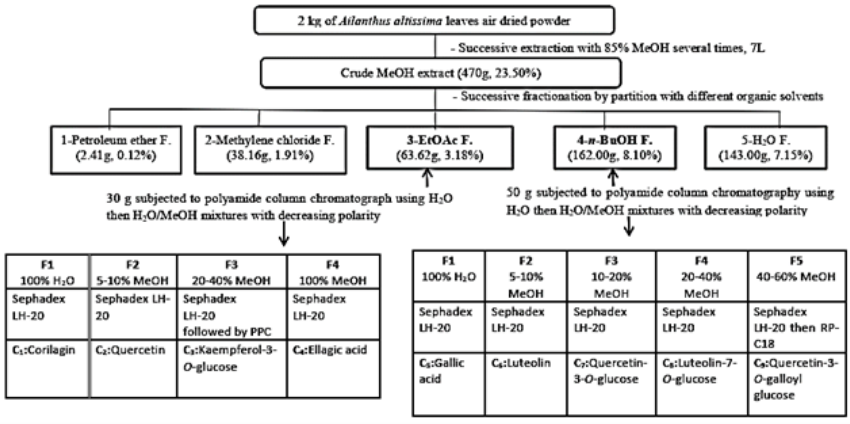


Figure 1. Schematic representation of extraction, fractionation and isolation of active constituents from *A. altissima* leaves

Equipment and conditions of spectroscopic analyses

NMR spectrometer

NMR spectrometer (Bruker Avance (III) NMR spectrometer, USA) 400MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. All analyzed compounds were dissolved in DMSO-d_6. The chemical shifts of the compounds' peaks were expressed in δ_{ppm} and their coupling constants (J) were expressed in Hz. The spectra were processed via MestReNova 6.0.2 software.

Thin layer chromatography hyphenated with mass spectrometer TLC-ESI-MS

A readymade silica gel TLC card was prepared by loading the purified compounds, previously dissolved in highly purified methanol, using clean capillary tubes. The prepared card was then subjected to the TLC-MS apparatus (Advion compact mass spectrometer (CMS) NY, USA), at Nawah Scientific, which eluted each spot, separately with methanol by the assistance of the pump that is attached to the device at a flow rate of 10 μL/min to 1 mL/min, into the ESI mass spectrometer. The mass spectra were detected in the ESI (negative mode) between m/z 100–1200. The peaks and the spectra were processed using the CheMass and Advion Data Express software.

High performance liquid chromatography hyphenated with photodiode array detector (HPLC-DAD)

The methanol extract, the ethyl acetate, the *n*-butanol derived fractions as well as the compounds isolated and purified from *A. altissima* fractions were dissolved in HPLC grade MeOH at known concentrations, filtered using a filter membrane (pore size 0.45 μm, Phenex, USA) and centrifuged for 5 min at 6000 rpm then injected to the HPLC apparatus; HPLC-DAD (LC-8A liquid chromatography system hyphenated with SPD-M20A photodiode array detector Shimadzu, Kyoto, Japan) with LC solution software. The chromatographic separation was performed through RESTEK (5 μm) RP-C18 analytical column (4.6× 150 mm) and the elution occurred at a flow rate of 1 mL/min. The solvent system consisted of gradient mixtures of methanol (B) and acidified water with 0.1 % formic acid (A) starting with 5% B at 0–5 min, 5–35% B at 5–25 min, 35–40 % B at 25–49 min, 40–46 % B at 49–55 min, 46–80 % B at 55–56 min and 80–100 % B at 65–65 min. The eluate was monitored at 210 and 280 nm for the detection of the major chemical constituents in the plant extract and both fractions. The injection volume was 40 μL and the total run time was 65 min. The R_t and the area under the peaks corresponding to the purified compounds were recorded and hence their concentrations were calculated in both the

crude extract and the fraction from which they were separated. The column was reconditioned for 10 min before each analysis. All chromatographic operations were carried out at room temperature.

Estimation of *in vitro* antioxidant potential

DPPH radical scavenging activity

The antioxidant potential of the purified compounds was evaluated through their ability to neutralize DPPH· (1, 1diphenyl-2-picryl hydrazyl radical) which is a stable dark purple radicle converts into yellow upon reduction. Different concentrations of the pure isolated compounds (60–1.25 μg/mL) in methanol were mixed with freshly prepared DPPH solution in methanol (0.09 mM) in a ratio of 1:1 according to method described by [16]. The reaction mixtures were allowed to stand for 30 minutes in dark at room temperature then the decrease in the optical density was measured at 517nm against blank. Ascorbic acid (AA) was used as a positive control. The scavenging ability of the isolated compounds toward DPPH· was calculated according to the following equation: DPPH· scavenging activity % = $(A_{control} - A_{sample}/ A_{control}) \times 100$ where $A_{control}$ is the absorbance of the control solution containing methanol instead of test sample while A_{sample} is the absorbance of the isolated compound. The determinations were carried out in triplicates and the results were expressed as SC_{50} which is the concentration of the isolated compound required to reduce half of DPPH·.

Statistical analysis

The statistical analyses were carried out using IBM SPSS Statistics (25) software. The results were expressed as means ± standard deviation (SD) and all experimental analyses were performed in triplicate.

Spectroscopic data of the isolated pure compounds

Corilagin (1′′-*O*-galloyl-3′′, 6′′-hexahydroxydiphenoyl-*β*-D-glucopyranoside) (1): White powder (300 mg). R_f values: 0.56 (S_1), 0.51 (S_2) and 0.57(S_3). Color UV dark purple. UV (MeOH): λ_{max} 209, 268 nm. $C_{27}H_{23}O_{18}$, negative ESI-MS *m/z* 633 $[M-H]^-$; MS^2 *m/z* 482 [M-H- galloyl moiety (152 amu)] $^-$, *m/z* 301 [M-H- galloylglucose (332 amu)]$^-$. ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 9.05 (9H, brs, OH), 7.02 (2H, s, H-2′′, 6′′), 6.57 (1H, s, H-3′), 6.50 (1H, s, H-3), 6.21 (1H, d, *J* = 7.2 Hz, H-1′′′), 5.82 (1H, br s, H-3′′′), 4.60 (1H, s, H-4′′′), 4.36 (1H, t, *J* = 8.1 Hz, H-5′′′), 4.24 (1H, dd, *J* = 11.0, 7.8 Hz, H-6′′′a), 3.95 (1H, dd, *J* = 10.7, 8.8 Hz, H6′′′b), 3.88 (1H, d, *J* = 7.3 Hz, H-2′′′). ^{13}C NMR (100 MHz, d_6- DMSO): HHDP δ_c 167.62 (C-7), 167.24 (C-7′), 165.35 (C-7′′), 146.00 (C-3′′, 5′′), 145.22 (C-4), 145.13 (C-6), 144.78 (C-4′), 144.44 (C-6′), 139.49 (C-4′′),

136.00(C-5), 135.83 (C-5′), 124.33 (C-2′), 123.62 (C-2), 119.16 (C-1′′), 116.24 (C-1), 115.91 (C-1′), 109.51 (C-2′′,6′′), 107.49 (C-3), 106.50 (C-3′), 92.73 (C-1′′′), 77.68 (C-5′′′), 76.71 (C-3′′′), 71.86 (C-2′′′), 64.37 (C-6′′′), 62.57 (C-4′′′).

Astragalin (kaempferol-3-*O*-*β*-D-glucopyranoside) (3): Green powder (78 mg). R_f values: 0.57 (S_1), 0.78 (S_2) and 0.58 (S_3). Color UV dark purple. UV (MeOH): λ_{max} (nm): 265, 348; NaOMe: 272, 325^{Sh}, 401; $AlCl_3$: 273, 303^{Sh}, 349, 398; $AlCl_3$/HCl: 271, 304^{Sh}, 348, 397; NaOAc: 274, 305^{Sh}, 368; NaOAc/ H_3BO_3: 265, 349. $C_{21}H_{19}O_{11}$, negative ESI-MS *m/z* 447 $[M-H]^-$; MS^2 *m/z* 285 [M-H- hexose unit (162 amu)]$^-$. ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 12.63 (1H, s, OH-5), 8.04 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.89 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 6.41 (1H, d, 1H, H-8), 6.20 (1H, d, H-6), 5.46 (1H, d, *J* = 6.1 Hz, H-1′′), 3.60-3.10 (6H, m, H-2′′,3′′,4′′, 5′′, 6′′). ^{13}C NMR (100 MHz, d_6- DMSO): δ_c 177.88 (C-4), 164.72 (C-7), 161.66 (C-5), 160.41 (C-4′), 156.80 (C-2, 9), 133.76 (C-3), 131.35 (C-6′), 122.06 (C-1′), 115.60 (C-5′), 104.38 (C-10), 101.33 (C-1′′), 99.18 (C-6), 94.04 (C-8), 77.94 (C-3′′), 76.92 (C-5′′), 74.55 (C-2′′), 70.33 (C-4′′), 61.38 (C-4′′).

Ellagic acid (4): Green powder (200 mg). R_f values: 0.068 (S_1), 0.57 (S_2) and 0.10(S_3). Color UV violet. UV (MeOH): λ_{max} 253, 368 nm. $C_{14}H_5O_8$, negative ESI-MS *m/z* 301 $[M-H]^-$. ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 7.44(2H, s, H-5, 5′). ^{13}C NMR (100 MHz, d_6- DMSO): δ_c 159.32 (C-7, 7′), 148.26 (C-4, 4′), 139.90 (C-3, 3′), 112.46 (C-1, 1′), 110.30 (C-5, 5′), 107.62 (C-6, 6′).

Gallic acid (5): Yellow powder (38 mg). R_f values: 0.61 (S_1), 0.89 (S_2) and 0.80(S_3). Color UV violet. UV (MeOH): λ_{max} 207, 271 nm. $C_7H_5O_5$, negative ESI-MS *m/z* 168.90 $[M-H]^-$. ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 6.92 (2H, s, H-2, 6); ^{13}C NMR (100 MHz, d_6- DMSO): δ_c 168.20 (C-7), 146.01 (C-3, C-5), 138.61 (C-4), 121.14 (C-1), 109.36 (C-2, C-6).

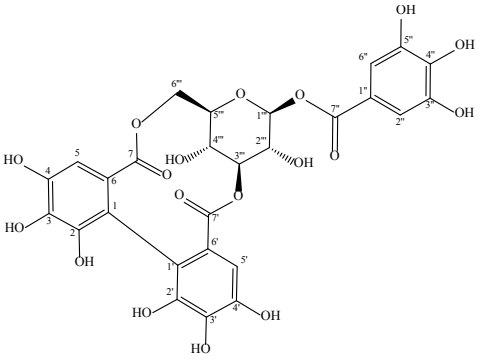
Isoquercetin (Quercetin-3-*O*-*β*-D-glucopyranoside) (7): Yellow amorphous powder (13 mg). R_f values: 0.54 (S_1), 0.76 (S_2) and 0.55 (S_3). Color UV dark purple. UV (MeOH): λ_{max} (nm) 256, 295^{Sh}, 354; NaOMe: 271, 325^{Sh}, 403; $AlCl_3$: 273, 303^{Sh}, 332, 433; $AlCl_3$/HCl: 271, 301^{Sh}, 355, 401; NaOAc: 270, 320^{Sh}, 375; NaOAc/ H_3BO_3: 265, 298^{Sh}, 375. $C_{21}H_{19}O_{12}$; negative ESI-MS *m/z* 463 $[M-H]^-$; MS^2 m/*z* 301 [M-H- glucosyl moiety (162 amu)]$^-$ and m/*z* 255 [M-H-glucosyl- CO_2-H]$^-$. ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 12.63 (1H, s, OH-5), 7.59 (1H, dd, H-6′), 7.57 (1H, d, *J* = 2.0 Hz, H-2′), 6.85 (1H, d, *J* = 8.2 Hz, H-5′), 6.40 (1H, d, *J* = 1.5 Hz, H-8), 6.18 (1H, d, *J* = 1.6 Hz, H-6), 5.45 (1H, d, *J* = 7.2 Hz, H-1′′), 3.58 (2H, d, *J* = 11.3 Hz, H-6′′), 3.35 (1H, d, *J* = 4.3 Hz, H-2′′), 3.32 (1H, d, *J* = 4.7 Hz, H-3′′), 3.24 (1H, d, *J* = 5.2 Hz, H-4′′), 3.09 (1H, s, H-5′′). ^{13}C

NMR (100 MHz, d_6- DMSO): δ_c 177.81 (C-4), 165.07 (C-7), 161.61 (C-5), 156.83 (C-2), 156.66 (C-9), 148.97 (C-4′), 145.27 (C-3′), 133.71 (C-3), 122.06 (C-6′), 121.55 (C-1′), 116.60 (C-5′), 115.71 (C-2′), 104.25 (C-10), 101.35 (C-1′′), 99.30 (C-6), 94.12 (C-8), 77.85 (C-3′′), 76.87 (C-5′′), 74.52 (C-2′′), 70.28 (C-4′′), 61.32 (C-6′′).

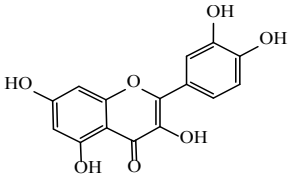
Cynaroside (luteolin-7-*O*-*β*-D-glucopyranoside) (8): Yellow powder (26 mg). R_f values: 0.23 (S_1), 0.76 (S_2) and 0.54 (S_3). Color UV dark purple. UV (MeOH): λ_{max} (nm) 255, 265^{Sh}, 295^{Sh}, 349; NaOMe: 265, 300^{Sh}, 404; $AlCl_3$: 270, 301, 425; $AlCl_3$/HCl: 273, 370, 390; NaOAc: 255, 365, 400; NaOAc/H_3BO_3: 255, 371. $C_{21}H_{19}O_{11}$; negative ESI-MS *m/z* 447 $[M-H]^-$; MS^2 m/*z* 285 [M-H- glucosyl moiety (162 amu)]$^-$. ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 12.98 (1H, s, OH-5), 9.66 (1H, s, OH-3′), 7.45 (1H, dd, *J* = 8.3 Hz, H-6′), 7.42 (1H, d, H-2′), 6.91 (1H, d, *J* = 8.3 Hz, H-5′), 6.79 (1H, d, H-8), 6.45 (1H, d, H-6), 5.08 (1H, d, *J* = 7.1 Hz, 1′′-H), 3.73 – 3.17 (6H, m). ^{13}C NMR (100 MHz, d_6- DMSO): δ_c 182.36 (C-4), 164.94 (C-7), 161.60 (C-5), 163.41 (C-2), 157.41 (C-9), 150.38 (C-4′), 146.24 (C-3′), 103.64 (C-3), 119.64 (C-6′), 121.86 (C-1′), 116.46 (C-5′), 114.03 (C-2′), 105.81 (C-10), 100.36 (C-1′′), 100.01 (C-6), 95.20 (C-8), 77.63 (C-5′′), 76.86 (C-3′′), 73.59 (C-2′′), 70.03 (C-4′′), 61.09 (C-6′′).

Tellimoside (quercetin-3-*O*-(6′′-*O*-galloyl)-*β*-D-glucopyranoside) (9): Brown powder (110 mg). R_f values: 0.43 (S_1), 0.80 (S_2) and 0.60 (S_3). Color UV dark purple. UV (MeOH): λ_{max} (nm) 263, 295^{Sh}, 354; NaOMe: 273, 326, 408; $AlCl_3$: 275, 304^{Sh}, 429; $AlCl_3$/HCl: 270, 300^{Sh}, 401; NaOAc: 272, 380; NaOAc/H_3BO_3: 265, 296^{Sh}, 375. $C_{28}H_{23}O_{16}$, negative ESI-MS *m/z* 615 $[M-H]^-$; MS^2 *m/z* 301 [M-H- glucosyl- galloyl moieties (314)]$^-$· ^{1}H NMR (400 MHz, d_6- DMSO): δ_H 12.55 (1H, s, OH-5), 9.23 (4H, s, OH-3′, 3′′′, 4′′′, 5′′′), 7.58 (1H, dd, *J* = 8.5, 2.0 Hz, H-6′), 7.44 (1H, d, *J* = 1.9 Hz, H-2′), 6.90 (2H, s, H-2′′′, 6′′′), 6.73 (1H, d, *J* = 8.5 Hz, H-5′), 6.38 (1H, d, *J* = 1.4 Hz, H-8), 6.19 (1H, d, H-6), 5.46 (1H, d, *J* = 7.1 Hz, H-1′′), 4.27 (1H, d, *J* = 11.2 Hz, H-6′′b), 4.18 (1H, dd, *J* = 11.7, 4.1 Hz, H-6′′a), 3.43 – 3.30 (4H, m, H-2′′,3′′,4′′,5′′). ^{13}C NMR (100 MHz, d_6- DMSO): δ_c 177.74 (C-4), 166.17 (C-7′′), 164.58 (C-7), 161.60 (C-5), 156.94 (C-2), 156.76 (C-9), 148.87 (C-4′), 145.86 (C-3′′,5′′), 145.22 (C-3′), 138.85 (C-4′′), 133.77 (C-3), 122.34 (C-6′), 121.42 (C-1′), 119.77 (C-1′′), 116.22 (C-5′), 115.74 (C-2′), 109.05 (C-2′′,6′′),104.35 (C-10), 101.73 (C-1′′), 99.19 (C-6), 94.04 (C-8), 76.71 (C-3′′), 74.68 (C-5′′), 74.49 (C-2′′), 70.01 (C-4′′), 63.60 (C-6′′).

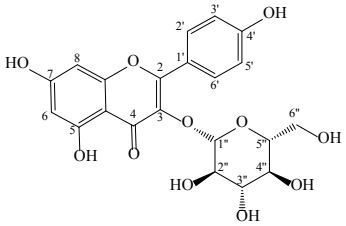
Two aglycones were separated, purified and identified based on the UV-Vis spectral analysis using different diagnostic shift reagents and co-PC, co-TLC with authentic sample which are quercetin (2, 30 mg) and luteolin (6, 30 mg).



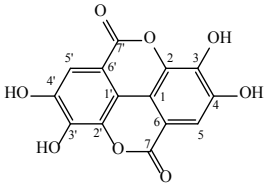
(1)



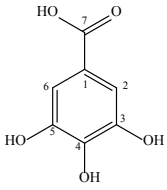
(2)



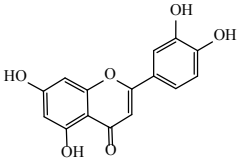
(3)



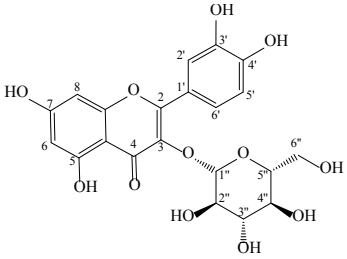
(4)



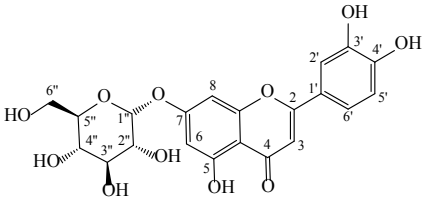
(5)



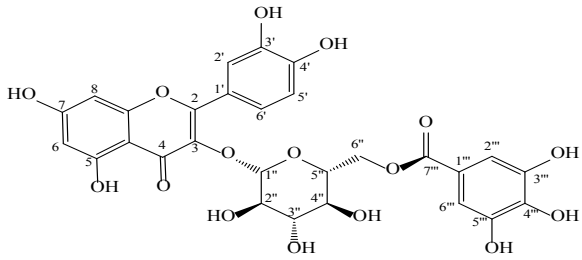
(6)



(7)



(8)



(9)

Figure 2. Isolated compounds from *A. altissima* leaves

Table 1. Pure compounds isolated from *A. altissima* leaves, their retention time and concentration in both methanol extract and derived fraction

Fraction	Compound	R_t(min)	Conc. (mg/g MeOH extract)	Conc. (mg/g fraction)
EtOAc fraction	Corilagin	16.24	5.138	19.17
	Ellagic acid	32.61	0.55	5.96
	Astragalin	34.75	40.85	133.23
	Quercetin	45.58	0.20	15.51
n-BuOH fraction	Gallic acid	7.31	68.96	26.86
	Tellimoside	24.12	19.29	20.744
	Cynaroside	26.16	3.62	1.73
	Isoquercetin	27.58	20.29	2.52
	Luteolin	51.01	0.15	0.18

Table 2. Pure compounds isolated from *A. altissima* leaves, their antioxidant potential via DPPH• scavenging activity

Compound	DPPH antioxidant activity (SC_{50} µg/mL)
Corilagin	4.91±0.032
Ellagic acid	2.26±0.027
Astragalin	11.28±0.044
Gallic acid	4.10±0.059
Tellimoside	9.52±0.036
Cynaroside	19.83±0.058
Isoquercetin	31.83±0.302
Ascorbic acid	6.44±0.03

The results are represented as mean of three analyses ± SD

Table 3. Biological properties of compounds isolated from *A. altissima* leaves

Cpd	Biological properties	Ref
Corilagin	Antimicrobial, antitumor, anti-inflammatory, antidiabetic, antihypertensive, hepatoprotective, neuroprotective, cardiovascular protective	17
Ellagic acid	Anti-allergic, anti-atherosclerotic, cardioprotective, hepatoprotective, nephroprotective, antitumor, neuroprotective	18
Astragalin	anti-inflammatory, neuroprotective, cardioprotective, antiobesity, anti-osteoporotic, anticancer, antiulcer, antidiabetic	19
Gallic acid	Anticancer, anti-inflammatory, cardiovascular protective, neuroprotective, antimicrobial, antiobesity, antidiabetic, hypolipidemic, gastroprotective	20
Tellimoside	lipid peroxidation inhibitor	21
Cynaroside	Anticancer, antimicrobial, anti-leishmanial, anti-inflammatory	22, 23
Isoquercetin	Anti-inflammatory, anticancer, cardioprotective, antidiabetic, anti-allergic, neuroprotective, antiviral	24-26

RESULTS AND DISCUSSION

Nine compounds were chromatographically isolated and purified from *A. altissima* leaves; four compounds from the ethyl acetate and five other compounds from the *n*-butanol fraction derived from the methanol extract of *A. altissima* leaves. Their chemical structures were elucidated based on the collection of different spectroscopic data and their comparison with the previously published one. Compound 1 was isolated as white powder that appeared as a dark purple spot on PC that did not change on spraying with $AlCl_3$.The deprotonated molecule ion $[M-H]^-$ appeared in the negative ion mode ESI-MS spectrum at *m/z* 633 which is consistent with the molecular formula of $C_{27}H_{23}O_{18}$. The molecule ion was further fragmented producing two MS^2 fragment ions at *m/z* 482 due to loss of galloyl moiety (152 amu) and at *m/z* 301 due to loss of galloylglucose (332 amu) which is a characteristic fragmentation pattern of ellagitannins [27]. The ^1H NMR spectrum (d_6- DMSO) displayed three singlet signals in the aromatic region; two singlets assigned for the two aromatic protons (3′, 3-H) of hexahydroxydiphenoyl (HHDP) which resonate at δ_H 6.50 and 6.57 ppm, respectively and one sharp singlet at δ_H 7.02 ppm for the 2′′ and 6′′ aromatic protons of galloyl moiety. Moreover, seven signals were observed for the protons of glucosyl unit; two singlets at δ_H 5.82 and 4.60 ppm due to protons attached to $C_{3'''}$, $C_{4'''}$, two doublets at δ_H 6.21 and 3.88 ppm due to protons attached to $C_{1'''}$, $C_{2'''}$ with coupling constant 7.2 and 7.3 Hz, respectively, pair of doublet of

doublet that resonate at δ_H 4.24 and 3.95 ppm due to protons attached to $C_{6'''a}$, $C_{6'''b}$ with coupling constant 11.0, 7.8 and 10.7, 8.8 Hz, respectively and finally one triplet signal at δ_H 4.36 ppm for 5′′′-H with coupling constant 8.1 Hz. The ^{13}C NMR spectrum of **compound 1** in d_6- DMSO exhibited 25 signals, 14 signals for the carbons of two benzene rings of HHDP, 6 signals for the glucose moiety and 5 signals for the gallic acid unit. Furthermore, the signals at δ_c 167.62, 167.24 and 165.35 are indicative to the three carbonyl groups of the two benzoyl units of HHDP and that of the galloyl moiety, respectively. Based on the chromatographic and spectral analyses as well as the reported data, **compound 1** was identified as corilagin [28, 29]. Corilagin was previously separated from *A. altissima* fruit [8] but it is the first time to be reported from the plant leaves.

Compound 2 was isolated as yellow amorphous powder that appeared on PC as a yellow spot that remained yellow on spraying with $AlCl_3$ and was identified as quercetin based on the UV-Vis spectral data, comparison with previous literature and co-PC, co-TLC with authentic sample [30]. Quercetin was isolated before from the leaves of *A. altissima* and *A. excelsa* (Roxb) [31, 32], respectively.

Compound 3 was isolated as green powder that appeared as a dark purple spot on PC that converted yellow on spraying with $AlCl_3$. The molecule ion peak $[M-H]^-$ appeared in the ESI-MS (negative mode) at *m/z* 447 which matched with the molecular formula of $C_{21}H_{19}O_{11}$. It was fragmented into a daughter peak of *m/z* 285 $[M-H-162]^-$ which indicated the loss of hexose unit The ^1H NMR (DMSO-d_6) exhibited two doublets at δ_H 6.20, 6.41 corresponding to 6-H, 8-H, respectively and one singlet at δ_H 12.63 ppm corresponding to 5-OH. Two doublets appeared in the HNMR at δ_H (ppm) 6.89 (d, *J* = 8.4 Hz, 2H) and 8.04 (d, *J* = 8.5 Hz, 2H) corresponding to two pairs of *ortho* coupled aromatic protons (3′, 5′-H and 2′, 6′-H, respectively). The anomeric proton of the glycosyl unit appear as doublet at δ_H 5.46 ppm with coupling constant 6.1Hz while the rest protons of the sugar unit appeared as multiplet in the aliphatic region at δ_H 3.60-3.10 ppm. The carbon signals of the aglycone part matched with the spectral literature of kaempferol. Moreover, the carbon signals of the sugar unit confirmed that it is *O-β*-D-glucopyranoside. The downfield shift of both $C\text{-}_2$ and $C\text{-}_4$ as well as the upfield shift of $C\text{-}_3$ proved that the glucose unit is attached to 3-OH of kaempferol aglycone. Based on the mentioned data and reported spectral analyses, **Compound 3** was identified as astragalin [33-35]. Astragalin was previously isolated and identified from *A. altissima* leaves [31].

Compound 4 was isolated in the form of green powder that appeared as a violet spot on PC that did not change on spraying with $AlCl_3$. The ^1H NMR of the compound dissolved in DMSO-d_6 exhibited one singlet signal at δ_H 7.44

ppm which is corresponding to two symmetrical aromatic protons. The ^{13}C NMR of the compound in DMSO-d_6 showed seven signals; one at δ 159.32 ppm corresponding to carbonyl carbon that appears down fielded due to *α, β*-unsaturated δ lactone, the six rest signals are for five aromatic quaternary carbons and one aromatic methine carbon at δ 148.26, 139.90, 136.47, 112.46, 107.62 and 110.30, respectively. Its deprotonated molecular ion peak $[M-H]^-$ appears at *m/z* 301 in the negative mode of ESI-MS**)** which is consistent with the molecular formula of $C_{14}H_5O_8$. From the supposed molecular formula and the ^1H and ^{13}C NMR spectral data the compound was identified as ellagic acid which is known by having C_2 plane of symmetry that divides the structure into two typical halves each is consisted of seven carbons, primed and unprimed carbon atoms in each half, have the same chemical shift. This data was in complete agreement with the previously published data by [36]. Ellagic acid was one of the phenolic acids existed in the leaves of *A. altissima* [37].

Compound 5 was obtained as yellow powder that appeared as a violet spot on PC which converted into dark violet on spraying with $AlCl_3$. The molecular ion peak appears at $[M-H]^-$ appears at *m/z* 168.90 in the ESI-MS (negative mode) which is conformity with the molecular formula of $C_7H_5O_5$. The ^1H NMR spectrum (DMSO-d_6) exhibited sharp singlet signal at δ 6.92 ppm which revealed the presence of two identical aromatic protons (H-2 and H-6). The ^{13}C NMR spectrum (DMSO-d_6) showed five signals corresponding to seven aromatic carbon atoms that resonate at δ_C (ppm); 109.36 (C-2, C-6), 121.14 (C-1), 138.61 (C-4), 146.01 (C-3, C-5) and 168.20 (C-7; carbonyl carbon). Compound **5** was identified as 3, 4, 5-trihydroxybenzoic acid (gallic acid) [38]. Gallic acid was previously reported in *A. altissima* leaves [37, 5] and *A. altissima* root bark [39].

Compound 6 was isolated as brown powder that appeared on PC as dark purple spot that turned yellow on spraying with $AlCl_3$. Compound **6** was identified as luteolin based on UV-Vis spectral data and comparison with published literature [40]. Luteolin was previously reported in *A. altissima* leaves [31, 41] and *A. excelsa* (Roxb) leaves [32].

Compound 7 was obtained as yellow amorphous powder that appeared as a dark purple spot on PC that turned yellow on complexing with $AlCl_3$ spraying agent. The deprotonated molecular ion peak $[M-H]^-$ appeared in the negative mode of ESI-MS at m/*z* 463 which is conformity with the molecular formula of $C_{21}H_{19}O_{12}$. It was further fragmented into two molecule ions of m/*z* 301 $[M-H-162]^-$ and 255 due to loss of glucosyl moiety (162 amu) and subsequent loss of a proton and a carbon dioxide molecule $[M-H\text{-glucosyl-}\ CO_2\text{-}H]^-$. The ^1H NMR of the compound (DMSO-d_6) showed a downfield singlet at δ_H 12.63

ppm attributed to 5-OH. Two *meta*-coupled protons appeared as doublets at δ_H 6.18 and 6.40 ppm with *J* 1.6 and 1.5 Hz corresponding to 6-H and 8-H. Also, the three aromatic protons of ring B appeared forming an ABX system at δ_H (ppm) 6.85 (d, *J* = 8.2 Hz, 1H, 5′-H), 7.57 (d, *J* = 2.0 Hz, 1H, 2′-H) and 7.59 (dd, 1H, 6′-H) which was overlapped with 2′-H. Moreover, a set of signals that appeared in the region δ_H 3.58- 3.09 ppm reflected the glycoside unit. The anomeric proton at δ_H 5.45 ppm which appeared as doublet with *J* 7.2 Hz together with the ^{13}C NMR chemical shifts of sugar unit carbons revealed that the sugar unit is *O-β*-D-glucopyranoside. The rest signals of ^{13}C NMR were in complete agreement with those of quercetin aglycone. The downfield shift of both C-$_4$ and C-$_2$ and the upfield shift of C-$_3$ in comparison with the aglycone indicated that the glucosyl moiety is attached to C-$_3$. **Compound 7** was identified as isoquercetin [42-44] which was previously separated from *A. altissima* fruit[8] but it is the first time to be reported in *A. altissima* leaves.

Compound 8 was separated as yellow powder that looked as a dark purple spot on PC that converted yellow on spraying with $AlCl_3$. The deprotonated molecular ion peak was exhibited at m/*z* 447 in the negative mode ESI/MS spectrum so that the molecular formula was $C_{21}H_{19}O_{11}$. It also showed a daughter ion peak at m/*z* 285 which suggested the loss of glucosyl unit (162 amu). The ^{1}H NMR (DMSO-d_6) exhibited the signals of luteolin skeleton with downfield shift of the two *meta*-coupled protons of ring A which resonated at δ_H 6.45 and 6.79 ppm for 6-H and 8-H, respectively revealing the glycosylation of 7-OH. A sharp singlet appeared at δ_H 6.75 ppm corresponding to 3-H of ring C. The presence of ABX system at δ_H (ppm) 6.91 (d, *J* = 8.3 Hz, 1H, 5′-H), 7.42 (d, 1H, 2′-H) and 7.45 (dd, *J* = 8.3 Hz, 1H, 6′-H), which was overlapped with 2′-H, characteristic to disubstituted B-ring. The anomeric proton of sugar unit appeared as doublet at δ_H 5.08 ppm with coupling constant 7.1 Hz revealing that the sugar unit is *β*-D-glucoside. The ^{13}C NMR (DMSO-d_6) displayed 21 signals with characteristic carbonyl carbon, four oxygenated carbons and two olefinic carbons at δ_C (ppm); 182.36 (C-$_4$), 163.41, 103.64 (C-$_2$ and C-$_3$), 164.94, 161.60, 150.38 and 146.24 for C-$_7$, C-$_5$, C-$_{4'}$ and C-$_{3'}$, respectively. The downfield shift of C-$_6$ and C-$_8$ as well as the upfield shift of C-$_7$ relative to the aglycone spectrum confirmed the glucosylation of 7-OH. Compound **8** was identified as Cynaroside [45- 47] that was separated before from the leaves and branches of *A. altissima* [31] and the leaves of *A. excelsa* (Roxb)[32].

Compound 9 was isolated as brown powder that appeared as a dark purple spot on PC that turned yellow on spraying with $AlCl_3$.The results of ESI-MS spectrum (negative mode) indicated a molecular ion peak $[M-H]^-$ at *m/z* 615

which is consistent with the molecular formula of $C_{28}H_{23}O_{16}$. The molecule ion was further fragmented producing a daughter ion at *m/z* 301 $[M\text{-}H\text{-}314]^-$ due to loss of glucosyl and galloyl moieties with 162 and152 amu, respectively. The ^{1}H NMR spectrum (DMSO-d_6) displayed a sharp singlet at δ_H 6.90 ppm attributed to the two aromatic protons of galloyl moiety. Furthermore, five signals were recorded in the aromatic region that were consistent with the skeleton of quercetin 12.55 (s, 1H, 5-OH), 9.23 (s, 4H, 3′-OH), 7.58 (dd, *J* = 8.5, 2.0 Hz, 1H, 6′-H), 7.44 (d, *J* = 1.9 Hz, 1H, 2′-H), 6.73 (d, *J* = 8.5 Hz, 1H, 5′-H), 6.38 (d, *J* = 1.4 Hz, 1H, 8-H) and 6.19 (d, 1H, 6-H). It also had a distinct doublet at δ_H5.46 ppm with *J* 7.1 Hz which is characteristic for the *β*-anomeric proton of glucose unit that is attached to C-$_3$ of quercetin. Also, a doublet at 4.27 ppm with *J* 11.2 Hz and a doublet of doublet at 4.18 ppm with *J* 11.7 and 4.1 Hz reflected the two protons of glucose methine group whose hydroxyl group is esterified with gallic acid. The rest protons of glucose unit appeared as a multiplet in the region from 3.43 to 3.30 ppm. The ^{13}C NMR spectrum (DMSO-d_6) contained 26 signals corresponding to 28 C- atoms; 15 carbons for the quercetin nucleus, 6 carbons for the glucosyl moiety and 5 carbons for the galloyl unit. The chemical shift of all signals is in complete agreement with reported data. The downfield shift of C-$_{6''}$ of glucose unit to 63.60 ppm and the upfield shift of its adjacent C-$_{5''}$ to 74.68 from their normal positions proved that the galloyl moiety is attached to the hydroxyl group of C-$_{6''}$ of glucose. Also, the downfield shift of the ortho carbon atoms (C-$_2$ and C-$_4$) to 156.94 and 177.74 ppm, respectively as well as the upfield shift of C-$_3$ of aglycone to 133.77 ppm indicated that the glucosyl unit is attached to the hydroxyl group at C-$_3$. Compound **9** was identified as tellimoside [48, 49]including the hitherto unknown flavonoids, kaempferol 3-O-β-(6″-galloylglucopyranoside that was previously reported in *A. altissima* leaves [5] and *A. altissima* fruit [8].

HPLC-DAD analysis was used to obtain a phytochemical profile of the methanol extract of *A. altissima* leaves, to confirm the purity of the isolated compounds and to identify their concentration in both the crude extract and the fraction from which they were isolated by knowing their retention time and the area under the peak in the obtained chromatograms hence the concentration of the injected pure samples as well as the crude extract and the fractions were known.

HPLC-DAD analyses of the crude extract and the pure isolated compounds were represented in table 1. Corilagin (200 *μ*g/mL) was found at 16.24 min and its concentration in the crude extract and the ethyl actate fraction could be 5.138 mg/g and 19.17 mg/g, respectively. Ellagic acid (200 *μ*g/mL) was found at 32.61 min and its concentration in the crude extract and the ethyl actate fraction could be 0.55 mg/g and 5.96 mg/g, respectively. Astragalin (1 mg/mL) was found at

34.75 min and its concentration in the crude extract and the ethyl actate fraction could be 40.85 mg/g and 133.23 mg/g, respectively. Quercetin (1 mg/mL) was found at 45.58 min and its concentration in the crude extract and the ethyl acetate fraction could be 0.20 mg/g and 15.51 mg/g, respectively. Gallic acid (1 mg/mL) was found at 7.31 min and its concentration in the crude extract and the *n*-butanol derived fraction could be 68.96 mg/g and 26.86 mg/g, respectively. Tellimoside (1 mg/mL) was found at 24.12 min and its concentration in the crude extract and the *n*-butanol fraction could be 19.29 mg/g and 20.74 mg/g, respectively. Cynaroside (1 mg/mL) was found at 26.16 min and its concentration in the crude extract and the *n*-butanol fraction could be 3.62 mg/g and 1.73 mg/g, respectively. Isoquercetin (1 mg/mL) was found at 27.58 min and its concentration in the crude extract and the *n*-butanol fraction could be 20.29 mg/g and 2.52 mg/g, respectively. Luteolin (1 mg/mL) was found at 51.01 min and its concentration in the crude extract and the *n*-butanol derived fraction could be 0.15 mg/g and 0.18 mg/g, respectively. The UV spectrum of each pure compound was also recorded which was in complete agreement with our previous UV-analyses and the previously reported data. According to the previously mentioned data, the most abundant phytochemicals in the ethyl acetate fraction are astragalin followed by corilagin and in the *n*-butanol fraction are gallic acid followed by tellimoside. Furthermore, the most predominant phytochemicals in the crude methanol extract are gallic acid followed by astragalin.

The antioxidant potential of the isolated pure compounds was also evaluated through DPPH$^{\cdot}$ scavenging technique. The results were presented in table 2. Ellagic acid exhibited the most potent antiradical activity followed by gallic acid and corilagin with IC_{50} values of 2.26±0.027, 4.10±0.059, 4.91±0.032 *µg/*mL against DPPH$^{\cdot}$, respectively which were stronger than ascorbic acid that scavenged DPPH$^{\cdot}$ with IC_{50} value of 6.44±0.03 *µg/*mL. The potent antioxidant potential of the ethyl acetate derived fraction could be attributed to its richness with ellagic acid and corilagin as well as astragalin which existed in considerable amount (133.23 mg/g fraction) and exhibited strong antioxidant activity (IC_{50} = 11.28±0.044 *µg/*mL). The strong antioxidant activity of the *n*-butanol derived fraction could be attributed to its content of gallic acid. Both ellagic acid and corilagin could be good candidates as potent natural anti-oxidative drugs against oxidative stress induced disorders. In addition to the antioxidant activity, the isolated phytochemicals were previously reported to possess many biological potential that are summarized in table 3 [17-26]. These versatile activities provide more evidence for the suitability of the plant derived natural products to serve as drugs and/or drug leads in the treatment of many maladies but more investigations for their pharmacological effects are required first.

STATEMENT OF ETHICS

Not required as no human participants or experimental animals were involved in the study.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

EAE, MME and ESA conceived and supervised the study. HRM performed the experimental work and wrote the first draft of the manuscript. All authors contributed to data analysis and interpretation, revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

FUNDING SOURCES

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

REFERENCES

1. Joshi BC, Sharma RP, Khare A. Quassinoids and Their Chemotaxonomic Significance. Int J Herb Med. 2013; 1(2): 200–203.

2. Al-Snafi AE. The Pharmacological Importance of *Ailanthus Altissima*-a Review. Int J Pharm Rev Res. 2015; *5*(2): 121–129.

3. Kim HM, Lee JS, Sezirahiga J, Kwon J, Jeong M, Lee D. A new canthinone-type alkaloid isolated from *Ailanthus altissima* Swingle. Molecules. 2016; 21(5): 1–10. https://doi.org/10.3390/molecules21050642

4. Mohamed HR, El-Wakil EA, Abdel-Hameed ES, El-Hashash MM, Shemis M. Evaluation of total phenolics, flavonoids, and antioxidant and cytotoxic potential of *Ailanthus altissima* (Mill.) Swingle leaves. J Rep Pharm Sci. 2021; 10(1): 130–136. https://doi.org/10.4103/jrpt-ps.JRPTPS

5. Albouchi F, Hassen I, Casabianca H, Hosni K. Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves. S Afr J Bot. 2013; 87: 164–174. http://dx.doi.org/10.1016/j.sajb.2013.04.003

6. Hong Z, Xiong J, Wu S, Zhu J, Hong J, Zhao Y. Tetracyclic triterpenoids and terpenylated coumarins from the bark of *Ailanthus altissima* ("Tree of Heaven"). Phytochem. 2013; 86: 159–167. http://dx.doi.org/10.1016/j.phytochem.2012.10.008

7. Wang R, Xu Q, Liu L, Liang X, Cheng L, Zhang M. Antitumour activity of 2-dihydroailanthone from the bark of *Ailanthus altissima* against U251. Pharm Biol. 2016; 54(9): 1641–1648. http://dx.doi.org/10.3109/13880209.2015.1110827

8. Ni J, Shi J, Tan Q, Chen Q. Phenylpropionamides, Piperidine, and Phenolic Derivatives from the Fruit of *Ailanthus altissima*. Molecules. 2017; 22: 1–12. https://doi.org/10.3390/molecules22122107

9. Jeong M, Kim HM, Ahn J, Lee K, Jang DS, Choi J. Chemico-Biological Interactions 9-Hydroxycanthin-6-one isolated from stem bark of *Ailanthus altissima* induces ovarian cancer cell apoptosis and inhibits the activation of tumor-associated macrophages. Chem-Biol Interact. 2018; 280: 99–108. https://doi.org/10.1016/j.cbi.2017.12.011

10. Yan Z-Y, Lv T-M, Wang Y- X, Shi S- C, Chen J-J, Bin-Lin. Terpenylated coumarins from the root bark of Ailanthus altissima (Mill.) Swingle. Phytochem. 2020; 175(2020): 112361. https://doi.org/10.1016/j.phytochem.2020.112361

11. Sánchez-Bonet D, García-oms S, Belda-antolí M, Padrón-Sanz C, Lloris-carsi JM, Cejalvo-Lapeña, D. RP-HPLC-DAD determination of the differences in the polyphenol content of *Fucus vesiculosus* extracts with similar antioxidant activity. J Chromatogr B. 2021; 1184(2021): 122978. https://doi.org/10.1016/j.jchromb.2021.122978

12. Simirgiotis MJ, Benites J, Areche C, Sepúlveda, B. Antioxidant capacities and analysis of phenolic compounds in three endemic *Nolan*a species by HPLC-PDA-ESI-MS. Molecules. 2015; 20: 11490–11507. https://doi.org/10.3390/molecules200611490

13. Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem. 2015; 30(1): 11–26. https://doi.org/10.1007/s12291-014-0446-0

14. Atta EM, Mohamed NH, Abdelgawad AAM. Antioxidants: an overview on the natural and synthetic types. Eur Chem Bull. 2017; 6(8): 365–375. https://doi.org/10.17628/ecb.2017.6.374-384

15. Li Z, Lan Y, Miao J, Chen X, Chen B, Liu G. Phytochemicals, antioxidant capacity and cytoprotective effects of jackfruit (*Artocarpus heterophyllus* Lam.) axis extracts on HepG2 cells. Food Biosci. 2021; 41(2021): 100933. https://doi.org/10.1016/j.fbio.2021.100933

16. El-Sayed MM, El-Hashash MM, Mohamed HR, Abdel-Lateef EE. Phytochemical Investigation and *in vitro* Antioxidant Activity of Different Leaf Extracts of *Salix mucronata* Thunb. J Appl Pharm Sci. 2015; 5(12): 80–85. https://doi.org/10.7324/JAPS.2015.501213

17. Li X, Deng Y, Zheng Z, Huang W, Chen L, Tong Q. Corilagin, a promising medicinal herbal agent. Biomed Pharmacother. 2018; 99(2018): 43–50. https://doi.org/10.1016/j.biopha.2018.01.030

18. Sharifi-Rad J, Quispe C, Castillo CMS, Caroca R, Lazo-Vélez MA, Antonyak H. Ellagic Acid: A Review on Its Natural Sources, Chemical Stability, and Therapeutic Potential. Oxid Med Cell Longev. 2022; 2022: 3848084. https://doi.org/10.1155/2022/3848084

19. Riaz A, Rasul A, Hussain G, Zahoor MK, Jabeen F, Subhani Z. Astragalin: A Bioactive Phytochemical with Potential Therapeutic Activities. Adv Pharmacol Sci. 2018; 2018: 9794625. https://doi.org/10.1155/2018/9794625

20. Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R. Pharmacological effects of gallic acid in health and diseases : A mechanistic review. Iran J Basic Med Sci. 2019; 22: 225-237. https://doi.org/10.22038/ijbms.2019.32806.7897

21. Enkhtuya E, Shimamura T, Kashiwagi T, Ukeda H. Antioxidative constituents in the leaves of *Paeonia anomala* grown in Mongolia. Food Sci Technol Res. 2017; 23(1): 63–70. https://doi.org/10.3136/fstr.23.63

22. Zou Y, Zhang M, Zhang T, Wu J, Wang J, Liu K. Antioxidant and anti-inflammatory activities of cynaroside from *Elsholtiza bodinieri*. Nat Prod Commun. 2018; 13(11): 1501–1504. https://doi.org/10.1177/1934578x1801301122

23. Tabrez S, Rahman F, Ali R, Alouffi AS, Akand SK, Alshehri BM. Cynaroside inhibits *Leishmania donovani* UDP-galactopyranose mutase and induces reactive oxygen species to exert antileishmanial response. Biosci Rep. 2021; 41(1): BSR20203857. https://doi.org/10.1042/BSR20203857

24. Valentová K, Vrba J, Bancířová M, Ulrichová J, Křen V. Isoquercitrin: Pharmacology, toxicology, and metabolism. Food Chem Toxicol. 2014; 68: 267–282. https://doi.org/10.1016/j.fct.2014.03.018

25. Orfali GC, Duarte AC, Bonadio V, Martinez NP, Melo ME, Araújo B. Review of anticancer mechanisms of isoquercitin. World J Clin Oncol. 2016; 7(2): 189–199. http://dx.doi.org/10.5306/wjco.v7.i2.189

26. Magar RT, Sohng JK. A Review on Structure, Modifications and Structure-Activity Relation of Quercetin and Its Derivatives. J Microbiol Biotechnol. 2020; 30(1): 11–20. http://dx.doi.org/10.4014/jmb.1907.07003

27. Dincheva I, Badjakov I, Kondakova V, Dobson P, McDougall G, Stewart D. Identification of the phenolic components in Bulgarian raspberry cultivars by LC-MS. Int J Agric Sci Res. 2013; 3(3): 127–138.

28. Nawwar MAM, Hussein SAM, Merfort I. NMR spectral analysis of polyphenols from *Punica granatum*. Phytochem. 1994; 36(3): 793–798. http://dx.doi.org/10.1016/S0031-9422(00)89820-9

29. Kochumadhavan A, Mangal P, Kumar LS, Meenakshi BM, Venkanna BU, Muguli G. Corilagin: First Time Isolation from the Whole Plant of *Phyllanthus maderaspatensis* L. Phar-

macog Commun. 2019; 9(4): 135–138. http://dx.doi.org/10.5530/pc.2019.4.28

30. Jain SA, Patel AD, Prajapati NK, Prajapti SP. Extraction of flavonoids of seed coat of *Bauhinia tomantosa*. Int J Chem Sci. 2011; 9(2): 657–663.

31. Jin M-I, Bae K-H, Chang H-W, Son, J-K. Anti-inflammatory compounds from the leaves of *Ailanthus altissima*. Biomol Ther. 2009; 17(1): 86–91. http://dx.doi.org/10.4062/biomolther.2009.17.1.86

32. Said A, Hawas UW, El-Shenawy S, Nofal SM, Rashed, K. Flavonoids and some biological activities of *Ailanthus excelsa* leaves. IUFS J Biol. 2010; 69(1): 41–55. http://dx.doi.org/10.18478/iufsjb.48603

33. Xiao ZP, Wu HK, Wu T, Shi H, Hang B, Aisa HA. Kaempferol and quercetin flavonoids from *Rosa rugosa*. Chem Nat Compd. 2006; 42(6): 736–737. http://dx.doi.org/10.1007/s10600-006-0267-3

34. Duc LV, Thanh TB, Huu TN. Terpenoids from *Dicliptera chinensis* (L.) Nees Grown in Vietnam and their Anti-inflammatory Activities. Asian J Biomed Pharm Sci. 2018; 8(64): 6–13. http://dx.doi.org/10.37285/ijpsn.2018.11.2.7

35. Rezende FM, Ferreira MJP, Clausen MH, Rossi M, Furlan CM. Acylated flavonoid glycosides are the main pigments that determine the flower colour of the Brazilian native tree *Tibouchina pulchra* (Cham.) Cogn. Molecules. 2019; 24(4): 718–734. http://dx.doi.org/10.3390/molecules24040718

36. Khallouki F, Haubner R, Hull WE, Erben G, Spiegelhalder B, Bartsch H. Isolation, purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of *Anisophyllea dichostyla* R. Br. Food Chem Toxicol. 2007; 45(3): 472–485. http://dx.doi.org/10.1016/j.fct.2006.09.011

37. Luís Â, Gil N, Amaral ME, Domingues F, Duarte AP. *Ailanthus altissima* (Miller) Swingle: A source of bioactive compounds with antioxidant activity. Bioresources. 2012; 7(2): 2105–2120. http://dx.doi.org/10.15376/biores.7.2.2105-2120

38. Ghareeb MA, Sobeh M, El-Maadawy WH, Mohammed HS, Khalil H, Botros S. Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato–renal protective potential against cyclophosphamide induced toxicity in mice. Antioxidants. 2019; 8(9): 4–7. http://dx.doi.org/10.3390/antiox8090415

39. Tan Q, Ouyang M, Wu Z. A new seco-neolignan glycoside from the root bark of *Ailanthus altissima*. Nat Prod Res. 2012; 26 (15): 1375–1380. http://dx.doi.org/10.1080/14786419.2011.587187

40. Youssef S. Flavonoids from *Onopordon heteracanthum* C.A. Mey. Bull Pharm Sci. 2003; 26(1): 83–89. http://dx.doi.org/10.21608/bfsa.2003.65472

41. Zhelev I, Georgiev K, Dimitrova-Dyulgerova I. Carotenoid profile of *Ailanthus altissima* stem bark, *in-vitro* antioxidant and antineoplastic activities. World J Pharm Res. 2016; 5(3): 1816–1825.

42. Liu H, Mou Y, Zhao J, Wang J, Zhou L, Wang M, et al. Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities. Molecules. 2010; 15(11): 7933–7945. http://dx.doi.org/10.3390/molecules15117933

43. Manivannan R, Shopna R. Isolation of quercetin and isorhamnetin derivatives and evaluation of anti-microbial and anti-inflammatory activities of *Persicaria glabra*. Nat Prod Sci. 2015; 21(3): 170–175.

44. Aisyah LS, Yun YF, Herlina T, Julaeha E, Zainuddin A, Nurfarida I. Flavonoid com-

pounds from the leaves of *Kalanchoe prolifera* and their cytotoxic activity against P-388 murine leukimia cells. Nat Prod Sci. 2017; 23(2): 139–145. http://dx.doi.org/10.20307/nps.2017.23.2.139

45. Chiruvella KK., Mohammed A, Dampuri G, Ghanta RG, Raghavan SC. Phytochemical and Antimicrobial Studies of Methyl Angolensate and Luteolin-7-*O*-glucoside Isolated from Callus Cultures of *Soymida febrifuga*. Int J Biomed Sci. 2007; 3(4): 269–278.

46. Iwashina T, Setoguchi H, Kitajima J. Flavonoids from the Leaves of *Vitex rotundifolia* (Verbenaceae), and their Qualitative and Quantitative Comparison between Coastal and Inland Populations. Bull Natl Mus Nat Sci Ser B Bot. 2011; 37(2): 87–94.

47. Delazar A, Nazemiyeh H, Afshar F, Barghi N, Esnaashari S, Asgharian P. Chemical compositions and biological activities of *Scutellaria pinnatifida* A. Hamilt aerial parts. Res Pharm Sci. 2017; 12(3): 187–195. http://dx.doi.org/10.4103/1735-5362.207199

48. Güvenalp Z, Demirezer LÖ. Flavonol Glycosides from *Asperula arvensis* L. Turk J Chem. 2005; 29(2): 163–169.

49. Nawwar M, Ayoub N, El-Raey M, Zaghloul S, Hashem A, Mostafa E. Acylated flavonol diglucosides from *Ammania auriculata*. Z Naturforsch C J Biosci. 2015; 70(1–2) c: 39–43. http://dx.doi.org/10.1515/znc-2014-4165